

§Appl. No. 09/937,100  
Amdt. dated January 25, 2005  
Reply to Office Action of, August 25, 2005

**Listing of Claims:**

Please **amend** the claims as follows:

**Listing of Claims:**

**Claim 1** (Cancelled)

**Claim 2** (Cancelled)

**Claim 3** (Cancelled)

**Claim 4** (Cancelled)

**Claim 5** (Cancelled)

**Claim 6** (Cancelled)

**Claim 7** (Cancelled)

**Claim 8** (Cancelled)

**Claim 9** (Cancelled)

**Claim 10** (Cancelled)

**Claim 11** (Cancelled)

**Claim 12** (Cancelled)

**Claim 13** (Cancelled)

**Claim 14** (Cancelled)

**Claim 15** (Cancelled)

**Claim 16** (Withdrawn) A method of screening a protein library comprising screening said library for one or more desired properties, followed by dereplication to identify one or more individual proteins in the library having the desired property.

**Claim 17** (Withdrawn) A method as claimed in claim 16 wherein the library is screened for binding to a target moiety.

**Claim 18 (Withdrawn)** A method as claimed in claim 17 wherein binding is detected by mass spectrometry, particularly matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) spectrometry.

**Claim 19 (Withdrawn)** A method as claimed in claim 16 wherein the library is screened for a specific biological activity.

**Claim 20 (Withdrawn)** A method as claimed in claim 17 wherein the target is a complex mixture, eg a mixture of molecules, whole cells or cell membranes.

**Claim 21 (Withdrawn)** A method of protein identification and/or sequencing comprising providing a library of individual proteins, one or more of which may bind to a target of interest, wherein each individual protein, together with its gene, is bound to an "associating moiety".

**Claim 22 (Withdrawn)** A method as claimed in claim 21 wherein the library of proteins is brought into contact with the target of interest either before or after the "associating moiety".

**Claim 23 (Withdrawn)** A method as claimed in claim 21 wherein after screening for binding to the target the library is dereplicated to identify one or more proteins with a desirable property, proteins which bind to the target.

**Claim 24 (Withdrawn)** A method as claimed in claim 21 where the Aassociating moiety@ is a particle.

**Claim 25 (Withdrawn)** A method as claimed in claim 24 wherein the particle is a latex bead.

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**Claim 26 (Withdrawn)** A method as claimed in claim 21 wherein the Associating moiety is a protein or protein complex.

**Claim 27 (Withdrawn)** A method as claimed in claim 26 wherein the "associating moiety" is avidin or streptavidin and each of the proteins in the library and their associated genes are biotinylated.

**Claim 28 (Withdrawn)** A method as claimed in claim 21 wherein the "associating moiety" is a bispecific binding molecule capable of binding to both the proteins and genes.

**Claim 29 (Withdrawn)** A method as claimed in claim 21 wherein the "associating moiety" is a living cell or cellular virus such as a bacteria or bacteriophage.

**Claim 30 (Withdrawn)** A method as claimed in claim 21 wherein one or other molecules which alter the properties of the proteins in the library are bound to the "associating moiety" .

**Claim 31 (Withdrawn)** A method as claimed in claim 21 wherein the genes encoding the proteins in the library are attached to the "associating moiety" prior to synthesis of the individual proteins.

**Claim 32 (Withdrawn)** A method as claimed in claim 21 wherein the library of proteins is a library of antibody proteins, eg a library of antibody domains such as Fvs.

**Claim 33 (Withdrawn)** A method of protein identification and/or sequencing comprising providing a library of individual proteins, one or more of which may bind to a target of interest, wherein each individual protein is attached to an individual "coding moiety".

**Claim 34 (Withdrawn)** A method as claimed in claim 33 wherein the "coding moieties" are particles with unique identifier "codes".

**Claim 35 (Withdrawn)** A method as claimed in claim 34 wherein the "codes" are different ratios of measurable signal, eg fluorescent, chemiluminescent or radioactive labels, or a physical feature such as a unique marking.

**Claim 36 (Withdrawn)** A method for analyzing mixtures of proteins comprising:

- (iii) digestion or cleavage of the protein mixture;
- (iv) fractionation of the resultant peptides; and
- (v) analysis of the resultant peptides by means of their mass and/or sequence.

**Claim 37 (Withdrawn)** A method as claimed in claim 36 wherein the fractionation in step (ii) is carried out using a library of protein binding agents.

**Claim 38 (Withdrawn)** A method as claimed in claim 36 wherein the resultant peptides are subjected to physical fractionation and/or chemical tagging as part of the fractionation step.

**Claim 39 (Withdrawn)** A method as claimed in claim 36 wherein the resultant peptides are subjected to addition of one or more amino acids as part of the fractionation step.

**Claim 40 (Withdrawn)** A method as claimed in claim 37 wherein the library of protein binding agents is a library of antibodies or antibody fragments.

**Claim 41 (Withdrawn)** A method as claimed in claim 37 wherein the protein binding agents are major histocompatibility proteins, T cell receptors and natural proteins or protein domains involved in protein-protein binding interactions, such as SH1 domains.

**Claim 42 (Withdrawn)** A method as claimed in claim 40 wherein the library of protein binding agents is pre-selected for binding to one or more proteins or peptides derived from the protein mixture or a related protein mixture under analysis.

**Claim 43 (Withdrawn)** A method as claimed in claim 42 wherein the protein mixture is derived from a normalised recombinant gene library.

**Claim 44 (Withdrawn)** A method as claimed in claim 36 wherein the protein mixture is initially bound to a solid phase prior to digestion or cleavage either via the N or C-terminus or via specific amino acids or via specific sequences of amino acids.

**Claim 45 (Withdrawn)** A method as claimed in claim 36 wherein specific amino acids or modified amino acids found in the proteins are derivatised prior to binding to a solid phase, such binding occurring either before or after digestion or cleavage of the protein mixtures.

**Claim 46 (Withdrawn)** A method as claimed in claim 45 wherein the specific, or modified amino acids are derivatised with biotin prior to binding to avidin or streptavidin.

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**Claim 47 (Withdrawn)** A method as claimed in claim 45 wherein specific, or modified, amino acids are derivatised with ligands prior to binding to ligand-specific affinity reagents.

**Claim 48 (Withdrawn)** A method as claimed in claim 36 wherein specific naturally modified amino acids found in the proteins are bound to a solid phase using modification specific affinity reagents, such binding occurring either before or after digestion or cleavage of the protein mixtures.

**Claim 49 (Withdrawn)** A method as claimed in claim 45 wherein more than one cycle of digestion/cleavage and derivatisation is carried out.

**Claim 50 (Withdrawn)** A method as claimed in claim 49 wherein mass analysis is carried out after each cycle of digestion or cleavage.

**Claim 51 (Withdrawn)** A method as claimed in claim 36 wherein peptides released after digestion/cleavage are fractionated using physical methods such as HPLC before or after fractionation using protein binding agents.

**Claim 52 (Previously Presented)** A library of individual proteins, one or more of which being able to bind to a target of interest, each of said proteins comprising within its amino acid sequence, or terminal to it, one or more individual identifier sequence amino acid tracts which are unique to said individual protein when bound to the specific target of interest, and are flanked by one or more protease sensitive sites, said library thus providing proteins comprising a diversity of said individual identifier sequence tracts.

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**Claim 53 (Previously Presented)** A library of claim 52, wherein said identifier sequence tracts have been generated randomly or semi-randomly.

**Claim 54 (Previously Presented)** A library of claim 52, wherein an individual protein of said library comprises multiple identifier sequence tracts.

**Claim 55 (Previously Presented)** A library according claim 54, wherein an individual protein comprises two adjacent identifier sequence tracts.

**Claim 56 (Previously Presented)** A library according to claim 52, wherein said protease sensitive site is a recognition site for endoprotease digestion.

**Claim 57 (Previously Presented)** A library of claim 56, wherein the recognition site is the site for enterokinase or Factor Xa.

**Claim 58 (Previously Presented)** A library according to claim 52, wherein said target of interest is a protein.

**Claim 59 (Previously Presented)** A library of claim 52, wherein the individual proteins of the library are antibodies or recombinant antibody domains, which comprise antibody variable regions.

**Claim 60 (Previously Presented)** A library of claim 59, wherein the antibody domain is an Fv domain consisting of a VH and a VL chain.

**Claim 61 (Previously Presented)** A library according to claim 60, wherein each chain has its own identifier sequence tract.

**Claim 62 (Previously Presented)** A library according to claim 59, wherein said identifier sequence tract is C-terminal to the Fv domain sequence.

**Claim 63 (Previously Presented)** A library according to 59, wherein the target of interest is an antigen.

**Claim 64 (Previously Presented)** A method of identifying a protein which binds to a target of interest, wherein the protein is a member of the library of claim 52, comprising:

- (i) bringing each of the individual proteins of the library comprising said one or more individual identifier sequence tracts and said protease sensitive site(s) in contact with one or more of said targets of interest under conditions for an individual protein of said library to bind to said target of interest to form a complex,
- (ii) isolating the complex formed by the individual protein and the target of interest,

**Claim 65 (Currently Amended)** A method of claim 64, further comprising:

- (iii) digesting said complex to cleave ~~the introduced~~ said protease sensitive sites releasing said individual identifier sequence tract(s), and
- (iv) determining said released sequence tract(s) by which said individual protein is finally identifiable.

**Claim 66 (Previously Presented)** A method of claim 64, wherein the individual protein has been encoded by a DNA construct comprising nucleic sequences coding for said one or more individual identifier sequence tracts and said protease sensitive site(s).



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**Claim 67 (Previously Presented)** A method of claim 66, wherein said DNA construct is part of a vector system.

**Claim 68 (Previously Presented)** A method according to claim 64, wherein the individual protein and target of interest bind in solution.

**Claim 69 (Previously Presented)** A method according to claim 64, wherein the complex of protein / target is isolated by removal of non-bound molecules.

**Claim 70 (Currently Amended)** A method according to claim 65 64 , wherein digestion of said complex is achieved by endoprotease.

**Claim 71 (Currently Amended)** A method according to claim 65 64, wherein determination of the released identifier sequence tract(s) is achieved by mass spectrometry.

**Claim 72 (Previously Presented)** A method of claim 71, wherein the mass spectrometry is MALDI-ToF.

**Claim 73 (Previously Presented)** A method according to claim 64, wherein the individual proteins of the library are antibodies or antibody domains having a variable region and the targets of interest are antigens.

**Claim 74 (Currently Amended)** A method of identifying a protein which binds to a target of interest wherein the protein is a member of the library as defined in claim 52, comprising the following steps:

(i) bringing each of the individual proteins of the library comprising said one or more individual

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identifier sequence tracts and said protease sensitive site(s) in contact or association with one or more of said targets of interest,

- (ii) isolating the complex formed by the individual protein and the target of interest,
- (iii) digesting said complex to cleave the introduced protease sensitive sites releasing said individual identifier sequence tract(s), and
- (iv) determining said released sequence tract(s) and using such sequence information to recover the individual protein library member from the library, and
- ~~(vi)~~ (v) including one or more further rounds of screening and enrichment for a protein which binds to the target of interest.